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# Effect of Gamma Radiation and Methanol-Acetone Extracts of Milk Fermented by *Streptococcus thermophilus* on the Survival of Salmonellae on Chicken Meat

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NOTE: THERE WERE NO PUBLICATIONS OR PATENTS AS A RESULT OF THIS RESEARCH.

The objective of this study was to assess the quantitative impact of an antimicrobial factor(s) produced by Streptococcus thermophilus on the survival and/or multiplication of Salmonella typhimurium especially when also subjected to ionizing radiation treatments. In addition the activity of the extract was assayed against several foodborne organisms.

## MATERIALS AND METHODS

### Cultures

S. thermophilus used in this study was obtained from the culture collection maintained in the Department of Food Science and Animal Industries, Alabama, A&M University, Normal, Alabama. The culture was propagated by subculturing in 9 ml of 10% sterile skim milk (1:9 w/v) on a weekly basis. All inoculated milk was incubated at 45°C.

The following test organisms were obtained from American Type Culture Collection. They included Enterobacter cloacae ATCC-23355, Escherichia coli ATCC-25922, Klebsiella pneumoniae ATCC-13883, Proteus vulgaris ATCC-13315, Pseudomonas aeruginosa ATCC-27853, S. typhimurium ATCC-14028, Salmonella anatum ATCC-9270, Salmonella enteritidis ATCC-9186, Salmonella dublin ATCC-15480, Serratia marcescens ATCC-8100, Staphylococcus aureus ATCC-25923, Staphylococcus aureus ATCC-3767, Staphylococcus epidermidis ATCC-12228, and Streptococcus pyogenes ATCC-19615. Staphylococcus aureus B-121, Staphylococcus aureus B124, and

Staphylococcus aureus 196E were obtained from the Eastern Regional Research Center stock culture collection.

Each organism was propagated by inoculation of 10 ml of tryptic soy broth (TSB) with a loopfull of culture from a slant and then incubated overnight at 35°C when 1 ml was used to inoculate 100 ml of TSB that was incubated for 18 h with agitation (150 rpm) at 35°C in a baffled Delong flask.

Fermentation and preparation of the methanol-acetone extract

A methanol-acetone extract of milk fermented by S. thermophilus was prepared as described by Sikes and Hilton (1987) and Pulusani et al. (1979). The product will be referred to as the MA extract.

Diffusion assay for antimicrobial activity of MA extract

The surface of tryptic soy agar (Difco) in a petri plate was inoculated with a five hr culture of each organism in tryptic soy broth (Difco) using a cotton swab (Bauer et al. 1966). A sterile filter paper disk of 12.7 mm diameter was impregnated with 0.050 ml of the MA extract and placed on the center of the seeded agar in each plate. The plates were then incubated at 35°C for 18 hr before the zones of inhibition were read with a vernier caliper. The reported zones of inhibition are the average of a horizontal and vertical measurement of each zone.

Various amounts of MA extract as indicated in Fig. 1 were added to 5.0 g amounts of mechanically deboned chicken meat (MDCM) inoculated with $10^{6.1}$ colony forming units (cfu) per g of

S. typhimurium ATCC 14028. Each sample was vacuum packed in a stomacher bag. One set of samples was analyzed immediately and the second set was incubated at 35°C for 24 h and then analyzed. Samples were stomached with 45 ml of sterile Butterfields buffer (Fisher). The number of cfu per ml were determined using standard pour plate techniques with Tryptic Soy Agar (Difco). Three petri plates containing 30 to 300 colonies each were counted using a New Brunswick Biotron II colony counter.

## RESULTS AND DISCUSSION

Addition of MA extract to inoculated MDCM resulted in an immediate small reduction in the number of cfu per g. Following incubation of the samples for 24 h it was discovered that the MA extract did not prevente the multiplication of S. typhimurium (Fig. 1). (The upper curve with squares represents the results after a 24 h incubation and the lower curve with dots the results obatained immediately after addition of the extract.) The slope of the two regressions were not significantly different. The regression equation for the samples analyzed immediately after addition of the extracts was Log ratio survivors = 0.043 - 1.764(ml of extract), where log ratio survivors = logarithm to the base 10 of N/No (number of surviving cfu) ÷ (original number of cfu).

R-square = 0.979.

Addition of MA extract to MDCM inoculated with $10^{9.38}$ cfu of S. typhimurium per g prior to its being treated with radiation significantly ($p<0.0001$) shifted the intercept of the regression

but there was no evidence of interaction between the radiation treatment and treatment with the extract (MA). The slopes of the two regressions in Fig. 2 were not different. (The regression described by the squares represents the results of irradiation of samples to which extract had been added.)

Addition of various amounts of the MA extract to 5.0 g amounts of MDCM resulted in the following changes in the pH of the meat: 0 ml, 6.71; 0.1 ml, 5.78; 0.2 ml, 6.09, and 0.3 ml, 4.94. For contrast, addition of the following amounts of reagent grade concentrated lactic acid to 5.0 g of MDCM resulted in these pH values: 0 ml, 7.00; 0.1 ml, 6.83; 0.2 ml, 6.67; and 0.3 ml, 6.59. It is unfortunately apparent that the pH changes in the meat produced by addition of the MA extract could easily account for any observation made in the studies described above.

All of the following test organisms were sensitive to the unneutralized MA extract: Enterobacter cloacae ATCC-23355, Escherichia coli ATCC-25922, Klebsiella pneumoniae ATCC-13883, Proteus vulgaris ATCC-13315, Pseudomonas aeruginosa ATCC-27853, S. typhimurium ATCC-14028, Salmonella anatum ATCC-9270, Salmonella enteritidis ATCC-9186, Salmonella dublin ATCC-15480, Serratia marcescens ATCC-8100, Staphylococcus aureus ATCC-25923, Staphylococcus aureus ATCC-3767, Staphylococcus epidermidis ATCC-12228, and Streptococcus pyogenes ATCC-19615. Staphylococcus aureus B-121, Staphylococcus aureus B124, and Staphylococcus aureus 196E. Of these organisms S. pyogenes was the most and S. aureus the least sensitive. Because the extract was not

neutralized it is uncertain that the inhibitory effects are due to the presence of an antibiotic substance.

Minimally, additional investigations will need to be completed with neutralized extracts and studies completed of the characteristics (sensitivity to protease) of this extract before any conclusions can be drawn.

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